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STUDIES ON THE CHEMISTRY AND FINE STRUCTURE OF ELASTIC FIBERS FROM NORMAL ADULT SKIN*

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ABSTRACT

Collagenase-prepared, purified elastic fibers from adult human skin consist of at least two morphologically distinct components, the unstained amorphous component, and internally-located, deeply staining microfibrils. Separation and isolation of the amorphous component was achieved by alkali preparation of the elastic fibers. Electron micrographs of alkali prepared fibers showed essentially amorphous component as most of the microfibrils had been solubilized. The amino acid composition of the amorphous component was that of classical elastin. The microfibrillar component embedded deep within the interstices of the fiber was separated by, first, removing the amorphous component with elastase, and subsequently solubilizing, with dithioerythritol (DTE), the cystine-containing microfibrils contained in the elastase-produced residue. The amino acid composition of this DTE-extracted microfibrillar material was similar to that of the peripheral microfibrillar component enzymatically removed from bovine fetal elastic fibers. Electron microscopic monitoring showed that only stained microfibrils remained after elastase digestion. A protein portion of the microfibrillar component was not solubilized by the DTE and was presumed to contain a low concentration of disulfide bonds. Studies on purified elastin (amorphous component) revealed that alanine is concentrated around desmosine crosslinks and that the pyrollidines are uniformly distributed along the elastin molecule thereby precluding a α-helix conformation. Dark field electron microscopy suggested that the desmosine crosslink region is dumbell-shaped and that the desmosine crosslinks are not equidistant from each other on the peptide chains.

It has been established by electron microscopy that mature elastic fibers, after staining with cationic lead and uranyl acetate, consist of a central non-staining amorphous core surrounded by stained tubular appearing microfibrils (1, 2). In a recent study on collagenase-prepared elastic fibers from *fetal* bovine ligamentum nuchae, the central amorphous component was separated from the peripheral microfibrillar component by solubilizing the cystine-containing microfibrils with the reducing compound, dithioerythritol (3). Partial characterization of the separated components showed that the amorphous component was the desmosine-containing protein, elastin, while the microfibrils consisted of protein(s) with an amino acid composition quite different from that of elastin.

In the present study on elastic fibers from normal *adult* human skin, it was noted that electron micrographs of such elastic fibers showed essentially no peripheral but only *internally-located microfibrils* in large numbers. This investigation reports on a) the separation of microfibril from amorphous component, b) the chemical nature of adult microfibrils in relation to those isolated from fetal elastic fibers, c) the distribution of some of the amino acids in alkali-prepared elastin (amorphous component) and d) the fine structure of acid-solubilized elastin examined by dark field electron microscopy.

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MATERIALS AND METHODS

Isolation and purification of elastic fibers. A non-hydrolytic collagenase method of Miller *et al.* (4) was used to isolate elastic fibers from normal adult human skin. Necropsy specimens of skin were obtained from the upper, outer thigh of male cadavers aged forty to seventy-two years. The specimens obtained from three different cadavers were processed separately and were not pooled at any stage. Fat and epidermis were mechanically removed from the skin. The remaining dermis, cut into small pieces, was finely minced in 3% Na_2HPO_4 with a Sorvall Omni-Mixer. Treatment with 25% KCl, 5 M gaunidine HCl and collagenase was carried out according to the method of Miller *et al.* (4).

A portion of the above prepared elastic fibers was further treated with an alkali method in which the fibers were suspended in 0.1 N NaOH at 98° C for one hour. Prior to this treatment the fibers had been left in acetone and then ether, 24° C, for 24 hours, respectively.

Electron microscopic monitoring of the collagenase-prepared and alkali-treated elastic fibers showed the former to contain both microfibrils and amorphous

component while the latter consisted of essentially amorpous component devoid of microfibrils.

Elastase digestion. Elastin preparations were incubated with electrophoretically purified elastase (Worthington Biochemical Corp.) in 0.1 M glycine buffer (pH 7.0) at 37° C for 48 hours. One mg of elastase was used for every 5 mg of elastin (4).

Solubilization of microfibrils from adult dermal elastic fibers. The residue remaining after elastase digestion of collagenase-prepared dermal elastic fibers was treated with the reducing agent, dithioerythritol (DTE), to solubilize the microfibrils by reduction of disulfide bonds. The reduction procedure is that of Ross and Bornstein (3). On dialysis, some of the solubilized material precipitated out. The still solubilized portion of microfibrillar component was designated the 'supernatant'.

Preparation of soluble elastin fractions. Soluble elastin was produced by partial acid hydrolysis of alkali-prepared elastin in boiling 0.25 M oxalic acid solution according to the method of Partridge, David and Adair (5). After treating the elastin with the oxalic acid solution in a boiling water bath for a half-hour, the solution was removed and water washings of the remaining insoluble elastin added to it. This was designated Extract 1. Fresh oxalic acid solution was added to the residual insoluble elastin and boiling was carried out for one hour. Extract 2 was then removed. Subsequently, every hour for four more hours the extracts with their washings were removed and fresh solution added. After 5½ hours the insoluble elastin had dissolved to form 6 extracts numbered 1 to 6. Extracts 4 and 5 which had a high α-elastin content, were each chromatographed at 8° C on a Sephadex G-100 column equilibrated with 0.05 M phosphate buffer containing 8 M urea. The material emerging in the void volume, the α-elastin, was selected for dark field electron microscopy.

The elastin fragments shown in Figures 7, 8, 9 were a gift from Dr. R. A. Anwar. This material, designated Dowex V in a recent publication by Shimoda, Bowman, Davis and Anwar (6), was obtained by applying an elastase digest of bovine ligamentum nuchae elastin sequentially to Sephadex G-25, cellulose phosphate and Dowex 50W columns. The desmosine-rich material from one column was fractionated further on the subsequent column to obtain the final high desmosine-containing fraction from the Dowex column designated Dowex V. The molecular size of the fragment, determined on a Sephadex G-50 column, was found to be approximately 6000.

Light and electron microscopy. Alkali-prepared elastin, of known amino acid composition, was fixed in formalin and processed as a histologic specimen. Staining was with Verhoeffs and New Orcein.

Bright field electron microscopy was used to examine purified samples of both enzyme-prepared and alkali-prepared elastin. The preparations were fixed for one hour in 2% osmium tetroxide buffered with s-collidine to pH 7.4. They were then dehydrated and embedded in epoxy resin. Sections were stained with azure 11-methylene blue for light microscopy. Thin sections were stained first, with uranly acetate and subsequently, with lead citrate. The sections were examined in a Philips 300 Electron Microscope.

Dark field electron microscopy, a dark field technique developed by Ottensmeyer (7) was used to examine solubilized fragments of elastin. Dark field conditions were achieved by tilting the electron beam. This technique appears useful for the study of the fine structure of unstained, unshadowed macromolecules of biological origin. For examination, the samples of solubilized elastin were dissolved in cold 0.01 M phosphate buffer, pH 7.0, at concentration of 1 ng/ml.

Chemical analyses. Amino acid analyses carried out by the Beckman-Spinco Auto analyser were performed on samples of elastin hydrolyzed in 6 N HCl *in vacuo* for seventy-two hours at 106° C. Desmosine and isodesmosine, respectively, were quantitatively determined on the analyser according to a procedure by Anwar (8). Microfibrillar material was hydrolyzed 24 hours.

Protein concentrations of the oxalic acid solubilized elastin fractions were determined by the method of Lowry using solubilized elastin from bovine ligamentum nuchae as the standard.

RESULTS

Chemical Studies

The amino acid composition of hot alkali extracted elastin from the thigh skin of a seventy-year-old man is shown in Table I. The composition is characteristic for elastin. An almost identical composition was obtained on elastin isolated from the skin of a forty-three-year-old and sixty-year-old subject, respectively. In the same table are given the amino acid compositions of the 6 fractions extracted with oxalic acid solution from insolubile elastin. There is a steady increase in the isodesmosine and desmosine concentrations from Extract 1 to 6 while certain amino acids such as proline, valine, leucine, phenylalanine and arginine remain relatively constant. Alanine shows a steady increase until in fraction 6, the alanine exceeds glycine in concentration. The aspartic acid and glycine tended to decrease in amount. The increase in concentration of the desmosines probably indicates progressive hydrolytic cleavage of peptide chains with proportionally less extraction of the desmosine linkage regions thereby leaving an increasingly more cross-linked elastin core. Certain amino acids had a constant concentration throughout the 6 fractions. Thus, proline + hydroxyproline give a value of 123 to 127 residues/1000 total residues in each fraction thereby suggesting a uniform distribution of pyrrolidines in the peptide chains. Alanine, from these data seems to be concentrated around the desmosine crosslinks. The relatively high concentration of aspartic acid, glutamic acid, serine and hydroxyproline in Extract 1 (the fraction with the lowest desmosine content) suggests that there is a small quantity of acidic amino acid-enriched peptides in elastin which may be distant from desmosine crosslinks. These acidic amino acids seem unlikely to be derived from contaminating microfibrillar material as the hydroxyproline is high and the arginine low. No hydroxylysine was present.

In Table II, the first column of figures gives the amino acid composition of the residue remaining after elastase digestion of collagenase-prepared elastic fibers from human dermis. The residue constituted 14.7 ± 0.2% of the weight of the

TABLE I

*Amino acid composition of adult dermal elastin and of the oxalic-acid solubilized products obtained from elastin**

Amino acid	Enzyme-prepared elastin	Alkali-prepared elastin	Oxalic-acid extracts of alkali-prepared elastin‡					
			1	2	3	4	5	6
Hydroxyproline	7.0	7.2	11.2	7.0	4.9	4.8	5.1	3.3
Aspartic acid	14.4	5.2	12.6	7.2	5.7	4.6	4.2	4.4
Threonine	16.0	7.0	9.2	7.1	6.9	6.8	7.6	8.2
Serine	14.2	6.3	13.6	10.3	7.5	7.3	7.7	9.1
Glutamic acid	27.9	18.8	38.1	25.5	23.2	22.4	22.2	24.0
Proline	103.0	120.0	115.0	117.0	118.0	118.0	120.0	124.0
Glycine	292.0	315.0	306.0	304.0	304.0	293.0	287.0	277.0
Alanine	215.0	249.0	232.0	252.0	259.0	274.0	277.0	282.0
Cystine/2	5.3	Trace	Trace	Trace	Trace	Trace	Trace	Trace
Valine	128.0	120.0	131.0	131.0	131.0	124	119	113
Methionine	2.4	1.2	0.8	Trace	Trace	0.2	0.6	1.2
Isoleucine	28.2	24.7	24.5	23.0	21.4	20.8	20.1	19.0
Leucine	62.8	61.4	50.7	50.9	56.5	55.8	56.0	57.2
Tyrosine	23.2	21.6	16.9	22.4	22.4	24.0	27.0	27.3
Phenylalanine	25.8	22.7	21.4	21.5	20.8	20.7	21.0	21.7
Isodesmosine/4	3.7	3.9	1.7	3.8	4.4	5.1	5.7	6.3
Desmosine/4	5.4	5.7	2.9	5.5	5.8	7.1	8.1	9.1
Lysinonorleucine/2	0.9	0.9	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
α-Aminoadipic acid§	2.0	—	—	—	—	—	—	—
Lysine	10.4	5.6	7.3	6.8	5.3	5.3	5.5	5.8
Histidine	1.6	Trace	0.5	0.3	0.2	0.2	0.2	0.5
Arginine	13.2	5.6	5.5	5.3	5.7	5.7	6.3	6.1

* Values are expressed as residues per 1000 residues. No correction was made for losses during hydrolysis. Figures are the average of 2 separate determinations on the sample. n.c. indicates 'not calculated'.

‡ Note that Extract 1 constituted 4.8% of the total protein extracted; Extract 2, 14.0%; Extract 3, 16.4%; Extract 4, 24.4%; Extract 5, 34.5%; and Extract 6, 5.8%.

§ As the α-aminoadipic acid was determined on performic acid-oxidized elastin, the 2.0 equivalents represent that of α-aminoadipic-δ-semialdehyde. Alkali destroys the semialdehyde and thus cannot be determined on alkali-prepared elastin.

elastic fibers in the two digestions performed on a given sample. Other samples from subjects aged forty-three and seventy gave similar results. The second column shows the composition of material solubilized by the reducing action of DTE on the residue remaining after elastase digestion of elastic fibers. Approximately 65% of this residue was solubilized by the DTE. The fact that 35% was not solubilized suggested that at least one other protein comprised the microfibril and that it probably had a low content of disulfide bonds. Both materials have an amino acid composition quite different from that of elastin. They contain a much higher concentration of polar (acidic and basic) and sulfur containing amino acids, less glycine and alanine and no desmosines and hydroxyproline. The values of the second column in Table II are quite similar to those of the third column. The third column represents the composition of the peripheral microfibrils solubilized by chymotrypsin from collagenase-prepared elastic fibers of *fetal* bovine ligamentum nuchae. Thus the elastase-produced residue and the DTE extract of it probably consist mainly of microfibrils with an amino acid composition surprisingly similar to that of fetal bovine microfibrils. The discrepancies in amino acid composition between columns 2 and 3 are probably due to the different sources of the elastic fibers, and to the differences in preparation of the microfibrils. In the case of adult human skin, elastase had to be used to digest away the amorphous component (elastin) as the microfibrils are located mainly within the interstices of the adult elastic fiber and not peripherally as in the fetus (see Fig. 2). The non-specific proteolytic activity of elastase probably partially digested the microfibrils thereby altering the amino acid composition before extraction with DTE. In addition, column 3 represents the composition of the total microfibrillar component (precipitate and supernatant after dialysis) while column 2 designates the supernatant fraction only. Approximately 33% of the DTE solubilized fraction precipitated during dialysis. The amino acid composition of the precipitate was different from that of the supernatant as demonstrated by Ross *et al.* (3).

Microscopy

Light microscopy. Figure 1 illustrates a stained preparation of alkali-prepared, purified dermal elastic fibers. Such fibers were the starting mate-

TABLE II

*Amino acid composition of the microfibrillar component from adult dermal elastic fibers**

Amino acid	Residue after elastase digestion of fibers‡	Reduced, dialyzed elastase-residue: supernatant§	Enzymatic digest of fetal calf microfibrils¶
Hydroxyproline	0	0	1.7
Aspartic acid	96.0	86.4	92.5
Threonine	68.5	51.0	47.3
Serine	78.4	54.4	52.8
Glutamic acid	91.7	93.5	98.3
Proline	45.7	91.2	73.5
Glycine	118.0	149.0	142.0
Alanine	79.2	113.0	82.6
Cystine/2	34.1	34.5	56.3
Valine	94.9	73.0	69.7
Methionine	10.2	6.6	13.0
Isoleucine	33.6	33.9	43.8
Leucine	78.5	62.6	65.5
Tyrosine	42.3	27.9	27.6
Phenylalanine	24.7	26.8	32.8
Isodesmosine/4	0	0	0
Desmosine/4	0	0	0
Lysine	25.9	35.2	36.7
Histidine	16.5	12.1	11.5
Arginine	59.3	48.1	42.3

* Values are expressed as residues per 1000 residues. Figures are the average of 2 separate determinations on the same sample.

‡ 'Residue' refers to the material remaining after digestion of collagenase-prepared, adult dermal elastic fibers with pancreatic elastase.

§ 'Supernatant' refers to material solubilized by DTE treatment of the residue remaining after elastase digestion of collagenase-prepared adult dermal elastic fibers. As some of the DTE-solubilized material precipitated out of solution during dialysis, 'supernatant' designates the material remaining in solution after dialysis.

¶ These values are from the work of Ross *et al.* (3). The material, soluble peptides, was obtained by digestion with chymotrypsin of collagenase-prepared elastic fibers from bovine, fetal ligamentum nuchae. Chymotrypsin selectively solubilizes the *peripheral* microfibrils of the elastic fiber.

rial for preparation of the soluble elastin. This photomicrograph demonstrates that the chemical and electron microscopic studies that followed were done on elastic-staining fibers corresponding to those seen on histologic examination of the skin. Under the light microscope stained collagenase-prepared elastic fibers appear similar to the alkali-prepared fibers shown in Figure 1.

Bright field electron microscopy. An electron micrograph of collagenase-prepared purified adult dermal elastic fibers is illustrated in Figure 2. Note the deeply stained microfibrils of transverse, longitudinal and oblique cuts lying embedded within the unstained amorphous component of the fiber. No peripheral microfibrils of the type described by Ross and Bornstein are present. Reduction with DTE does not alter the electron micrographic appearance of these elastic fibers.

Figure 3 is an electron micrograph of a longitudinally oriented, collagenase prepared elastic fiber showing branched, interlacing microfibrils approximately 110 μ in diameter. The beaded appearance of the microfibrils is probably due to their wavy character.

Figure 4a shows a collagenase-prepared elastic fiber with the internally located microfibrils cut transversely. Figure 4b demonstrates the appearance of an elastic fiber prepared with alkali. Note the decrease in the number of internally-located microfibrils and the fuzzy outline and reduced stainability of the remaining ones. The sodium hydroxide treatment has solubilized most of the microfibrillar component. Alkali-preparation of elastic fibers, therefore, yields essentially the amorphous component.

Figure 5 is an electron micrograph of the residue remaining after elastase treatment of collagenase-prepared elastic fibers. Only cationic lead- and uranyl acetate-stained microfibrils are present as the amorphous component, the elastin, has been solubilized by the elastase. The general shape of the elastic fiber has been retained but owing to the absence of the elastin the microfibrils are crowded together. Discrete, round, transversly-cut microfibrils are clearly visible. The amino acid composition of this substance(s), the starting material for the DTE extraction, is given in the first column of values in Table II.

Dark field electron microscopy. Extracts 4 and 5 were examined by the dark field technique as the high desmosine and relatively low polar amino acid content indicated a preparation of solubilized elastin uncontaminated with microfibrillar component. In fact, only the α-elastin component of each of these extracts was used for dark field electron microscopy. The molecular size of the α-elastin, determined on a Sephadex G-200 column, was approximately 80,000.

Figure 6 shows a typical field consisting of electron dense chains 10 to 25 Å thick on which nodes 30 to 40 Å in diameter were located. The nodes from which 2 to 3 or occasionally even 4 chains radiated *may* represent the location of desmosine crosslinks. The internodal distances varied from 50 to 150 Å. Many of these chains and nodes were observed to form a lattice-like arrangement (Fig. 6). The sizes of the lattice-like structures varied considerably demonstrating the polydisperse nature of α-elastin. The electrophoretically-homogeneous elastase-produced peptide of 6000 molecular size, isolated from bovine ligamentum nuchae, was also examined by this technique. Figure 7 is a photomicrograph of this peptide showing a dumbell-shaped electron dense shape. Two electron dense circles were joined by a short crossbar. In Figure 8 another field, a similar structure was present. In Figure 9 a third field examined, there

FIG. 1. (Top) Light micrograph of purified elastic fibers isolated from adult human dermis. × 1200 (Verhoeffs Stain).

FIG. 2. (Bottom) Electron micrograph of collagenase-prepared, dermal elastic fibers. Note the large number of dark-staining microfibrils located internally within the elastic fibers but no periperal microfibrils. × 8000.

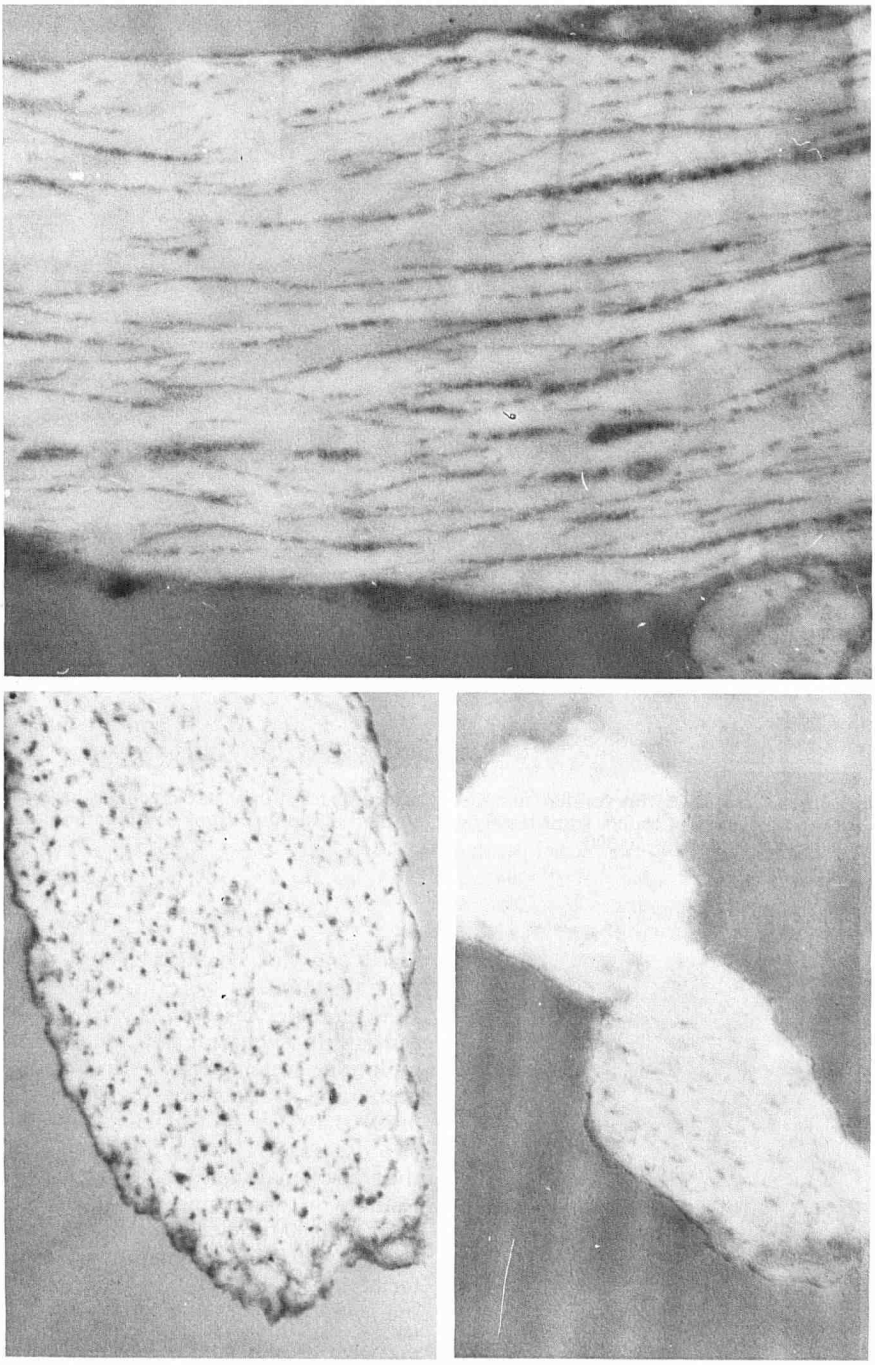

FIG. 3. (Top) Electron micrograph of a collagenase-prepared, dermal elastic fiber longitudinally oriented. The beaded, branching nature of the stained microfibrils is apparent. × 31,900.

FIG. 4. (Bottom) (a) Electron micrograph of a transverse section of a collagenase-prepared elastic fiber. × 27,700. (b) Electron micrograph of a transverse section of an alkali-prepared elastic fiber. Note the marked decrease in the number and stainability of the microfibrils. × 27,400.

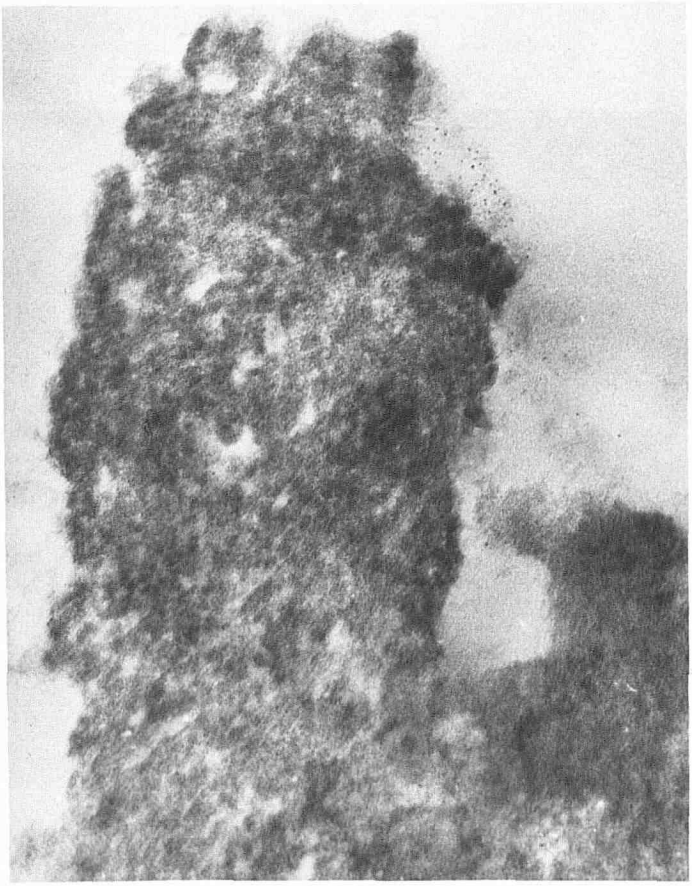

FIG. 5. Electron micrograph of the residue remaining after elastase digestion of collagenase-prepared dermal elastic fibers. Note the presence of round, transversely cut, stained microfibrils crowded together and the absence of unstained amorphous component. × 59,600.

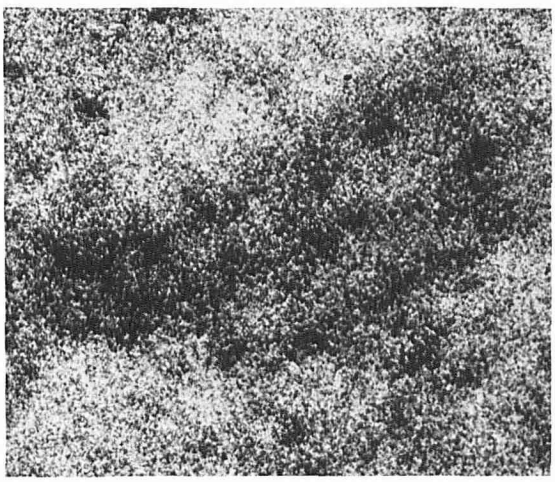

FIG. 6. Dark field electron micrograph of solubilized insoluble elastin (α-elastin) showing a lattice of electron-dense chains and nodes. The dotted background is produced by the carbon coating on the grid. × 700,000.

was a group of 'dumbell' structures with no crossbars clustered together in the center of the field. Here pairs of electron-dense circles appear tangential. In such fragments of elastin, each 6000 molecular weight in size and known to contain desmosine, or isodesmosine there is a high probability that a given fragment contains just 1 mole of a desmosine. This probability exists because in the source material, insoluble elastin, there are 10 residues of desmosine per 1000 amino acid residues (100,000 molec. wt). In the fields examined, the sizes of these fragments were remarkably uniform.

DISCUSSION

Like fetal bovine elastic fibers, elastic fibers from adult human skin consist of at least two components observable with the electron microscope. The main component, the unstained amorphous matrix, was chemically separated from the second component and found to have the amino

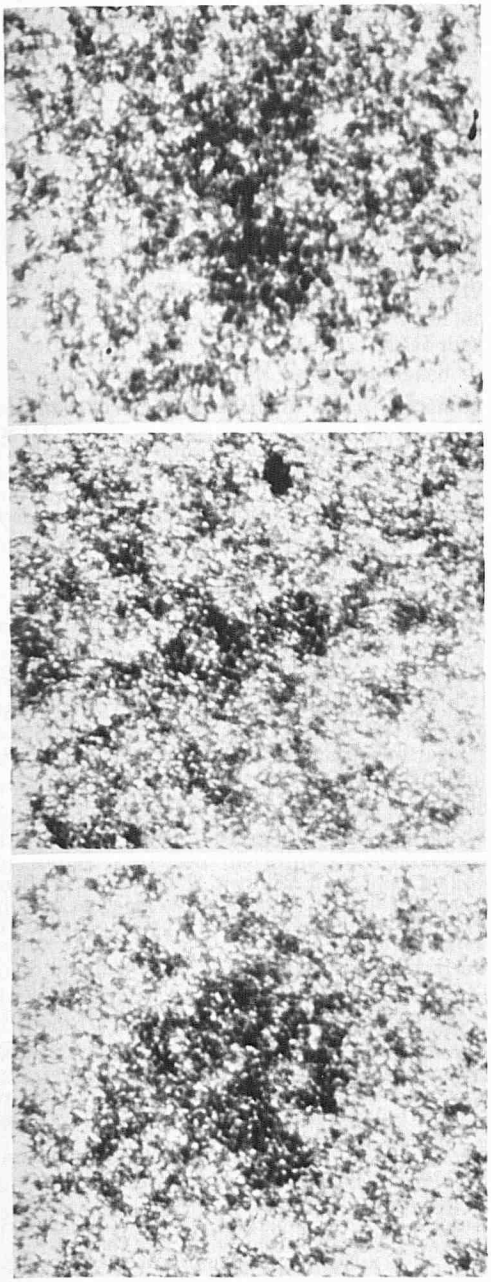

FIG. 7. Dark field electron micrograph of a desmosine-containing 6000 mol. wt. peptide derived from insoluble elastin of bovine origin. Note the electron-dense dumbell shaped structure. × 4,000,000.

FIG. 8. Dark field electron micrograph showing another field on the material illustrated in Fig. 7. Two ring or annular structures tangential to each other are demonstrated. × 4,000,000.

FIG. 9. Dark field electron micrograph showing another field of the material in Fig. 7. Note the cluster of paired ring structures with the 2 rings comprising a pair in tangential contact with each other. × 4,000,000.

acid composition of classical elastin. The second component, the stained microfibril, was found to constitute, at minimum, 15% of the dry weight of the adult dermal elastic fiber compared with 5–10% (3) of the fetal bovine elastic fiber. In addition, the microfibrils in the human adult elastic fiber were found to be mainly internal within the elastic fiber and not peripheral to it. The essential lack or small quantity of peripheral microfibrils probably indicated that there is little *de novo* synthesis of elastic fibers in the adult. The internally-located microfibrils are likely, therefore, historic markers of previous synthetic activity in which the diameter of the elastic fiber was constructed concentrically much like the Haversian units in bone. The diameter of adult elastic fibers is larger than that of fetal fibers and the half-life of elastin in the adult is probably long. It has been suggested that the laying down of microfibrils is the initial step in the synthesis of an elastic fiber (2). The chemical nature of the microfibrillar component, as determined by the amino acid composition, is similar to that from fetal bovine elastic fibers. This latter finding suggests that the microfibril is found generally in the connective tissues of mammals. It probably, therefore, represents a highly successful evolutionary feature of mammalian connective tissue. Ross *et al.* have suggested that one function of the microfibril is to determine the shape of the elastic fiber (3).

Alkali-prepared elastic fibers demonstrate a greatly reduced number of internal microfibrils on electron microscopy (Fig. 4b). Thus, such fibers should be a purer preparation of elastin (amorphous component) than collagenase-prepared fibers. This finding is supported by the amino acid composition of alkali prepared elastic fibers in comparison to enzyme prepared ones (Table I). The constancy in amino acid composition of alkali-prepared elastin may be similarly explained. The alkali-prepared elastic fibers consistently contain a much lower concentration of polar and sulfur-containing amino acids, the ones present in high concentration in the microfibrillar component. Thus, there was correlation between the electron microscopic appearance of the elastic fibers and their chemical composition. It appears now, that this microfibrillar protein(s), an integral part of the elastic fiber, was the reason for the difficulty in obtaining purified elastin free of 'non-collagen contaminant' even with alkali preparation.

Treatment of collagenase-prepared adult dermal elastic fibers with DTE did not alter their appearance with the electron microscope. The internally located microfibrils were as strongly stained and as sharp as before. In some recent unpublished work from this laboratory, it was found that collagenase-prepared elastic fibers from human *fetal* dermis contained a very high

concentration of polar and sulfur-containing amino acids. This suggested a higher percent of microfibrillar component in human fetal dermal elastic fibers than in the corresponding adult fibers. Such a conclusion was supported by the electron microscopic appearance of 22-week fetal fibers showing masses of peripheral microfibrils surrounding a small amorphous core that contained few internal microfibrils.

Amino acid analyses on the soluble elastin fractions (solubilized amorphous component) demonstrated an increasing desmosine content due to the probably artefactual creation of progressively more highly crosslinked peptides. The greater the enrichment of the peptide with desmosine, the higher was the alanine concentration. Reports by Shimuda *et al.* (6) and Keller *et al.* (9) have also demonstrated alanine enrichment around desmosine crosslinks in mature elastin from bovine ligamentum nuchae. Sandberg *et al.* (10) have isolated relatively small peptides containing 3 and 4 moles of alanine, respectively, to 1 mole of lysine, from 'tropoelastin' (soluble elastin from copper deficient animals). There is, however, some controversy whether 'tropoelastin' is a precursor of mature elastin. The constant value of the pyrrolidines (proline+hydroxyproline) in the various fractions suggests but does not prove a uniform distribution of this compound throughout the elastin protein molecule. Thus approximately every 8th amino acid is probably a pyrrolidine, a situation which likely precludes α-helix conformation. Phenylalanine, lysine and arginine also appear to be relatively uniformly distributed along elastin polypeptides. The high concentration of acidic amino acids in very low desmosine crosslinked peptides suggests that acid enriched portions of the polypeptide chains exist in elastin and that they are probably distantly located from the crosslinks.

The fact that α-aminoadipic-δ-semialdehyde is present in adult dermal elastic fibers (Table I) suggests that synthesis of elastin is still in progress. It probably indicates *de novo* synthesis and not merely increased crosslinking of pre-existing elastin. An alternative, however, is that some α-aminoadipic-δ-semialdehyde residues are not converted to crosslinks such as the desmosines.

The dark field electron microscopy suggests that the desmosine, if represented by the electron dense nodes, may not be uniformly distributed along the peptide chains as the internodal distances vary from 50 to 150 Å. The desmosine linkage region appears to be dumbbell-shaped with or without a short crossbar between the rings. This shape was repeatedly observed in the many fields examined. Three such fields (Figs. 7, 8, 9) are illustrated in the paper. One may speculate that the electron dense region between the rings represents the desmosine or isodesmosine while the rings themselves represent peptide clouds. The width of the circumferential line enclosing a ring correlates well with the diameter of parallel-oriented, contiguous peptide chains. This technique was used recently to verify the helical structure of DNA filaments and to reveal a U-shape for ribonuclease and a clover-leaf form for transfer RNA (7).

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